Time-Dependent Toxicity and Behavioural Alterations in Oreochromis mossambicus Exposed to Monocrotophos: A Quantitative Assessment

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ABSTRACT

Monocrotophos (MCP) is one of the more heavily used organophosphate insecticides in global agriculture, and its runoff into adjoining water bodies remains a persistent source of ecological stress for non-target aquatic organisms. A large part of the regulatory toxicology literature, however, still leans on the standard 96-hour LC_{50} bioassay - a design that was never intended to capture what happens when fish are exposed to pesticide residues for days or weeks at a stretch, as is typical of contaminated watersheds. This mismatch raises the possibility that acute assays systematically understate the real hazard. To examine this, static-renewal bioassays were carried out on Mozambique tilapia (*Oreochromis mossambicus*) across two exposure windows - 7 days (nominal MCP range 0-10.0 ppm) and 14 days (0-4.5 ppm). Probit analysis placed the 7-day LC_{50} at 3.6 ppm (95% CI: 3.2-4.1 ppm) and the 14-day LC_{50} at 2.4 ppm (95% CI: 2.1-2.7 ppm) - a 33% drop in tolerance that the non-overlapping confidence intervals confirm is statistically meaningful rather than incidental. Behavioural monitoring, scored quantitatively across replicate tanks, showed a fairly consistent progression: early hyperactivity within the first 24-48 hours, followed by loss of equilibrium and erratic swimming, and finally respiratory distress in the later stage of exposure (days 7-14). At 3.0 ppm, for instance, 85% of surviving fish were swimming erratically and 75% had lost normal balance by day 14. Taken together, the widening toxic response over time is consistent with cumulative acetylcholinesterase (AChE) inhibition outpacing the fish's capacity for metabolic detoxification and enzyme turnover. These results argue for building extended-duration exposure protocols and behavioural endpoints into the risk assessment framework used for organophosphate insecticides, rather than relying on acute lethality data alone.

Keywords: Monocrotophos, Organophosphate insecticide, LC_{50}, *Oreochromis mossambicus*, Behavioural toxicology, Probit analysis, Aquatic ecotoxicology

INTRODUCTION

Pesticide runoff into freshwater systems is one of the more intractable consequences of intensive agriculture, and it continues to pose a serious challenge to the ecological integrity of aquatic habitats worldwide [1, 2]. Organophosphate insecticides occupy an outsized share of this problem. Despite mounting evidence of their non-target toxicity, they remain a mainstay of pest control across tropical and subtropical farming systems, largely because they combine broad-spectrum efficacy with a cost advantage that few alternatives can match [3].

Field data collected in recent years make clear that this is not a legacy concern. Monocrotophos and related organophosphates continue to turn up in agricultural watersheds across Asia and Africa: water samples from intensively cultivated vegetable belts in Tamil Nadu, for instance, have shown residues as high as 0.5 µg/L [4], and comparable contamination patterns have been reported in surface waters in Thailand, Indonesia, and Nigeria [5, 6]. The persistence of these findings is itself an argument for revisiting toxicological baselines that were largely established decades ago, under exposure assumptions that may no longer reflect field reality.

Monocrotophos, chemically dimethyl E-1-methyl-2-methylcarbamoylvinyl phosphate (CAS No. 6923-22-4) - is applied widely against sucking and chewing pests in cotton, rice, and vegetable cultivation [7, 8]. Its toxicity stems from irreversible inhibition of acetylcholinesterase (AChE), the enzyme that normally terminates synaptic signalling at cholinergic junctions [9, 10]. Once AChE activity is blocked, acetylcholine accumulates at nerve endings and cholinergic receptors are driven into a state of continuous stimulation, producing the cascade of neuromuscular dysfunction, behavioural disturbance, and - at sufficiently high exposure - death that characterises organophosphate poisoning [11, 12]. Because this mechanism is well characterised, AChE inhibition has become a standard biomarker in fish toxicology, offering a direct biochemical link between exposure and the behavioural or lethal endpoints typically measured in bioassays [13, 14].

The ecological concern does not end with direct mortality. Organophosphate residues persist long enough in aquatic systems to accumulate through the food chain, raising the stakes for species at multiple trophic levels [15, 16]. Monitoring in agricultural catchments has repeatedly picked up monocrotophos in surface waters and irrigation canals, with concentrations peaking predictably after monsoon-driven runoff [17, 18] - a pattern that argues strongly for laboratory assays designed around realistic, field-relevant exposure durations rather than a single acute pulse. Fish are a natural sentinel for this kind of contamination, and among freshwater species, *Oreochromis mossambicus* (Mozambique tilapia) has become something of a workhorse model in ecotoxicology. Its broad tropical and subtropical distribution, physiological hardiness, tolerance of variable water quality, and ease of laboratory husbandry make it a practical and reproducible choice for toxicity testing [20, 21].

Rationale of the Study

Most regulatory toxicity testing still defaults to the 96-hour LC_{50} assay [22]. It is a well-standardised protocol, but it was designed to answer a narrower question than the one agricultural runoff actually poses, and three limitations in particular stand out:

Temporal mismatch- Real-world pesticide exposure in agricultural watersheds tends to be prolonged and low-grade rather than a single acute spike - the opposite of what a 96-hour test is built to measure [23].

Cumulative toxicity-longer exposure windows give time for bioaccumulation, sustained AChE inhibition, and the gradual exhaustion of detoxification capacity - processes that simply cannot register within four days [24, 25].

Sublethal blind spots- Mortality alone says nothing about the behavioural and physiological impairments that occur well below the LC_{50}, yet these can still be significant enough to compromise a fish's fitness in the wild [26, 27].

Behavioural endpoints help close that last gap. Because they register neurotoxic stress at sublethal concentrations, they are arguably a more ecologically meaningful signal than mortality alone [28, 29]. Changes in swimming pattern, loss of equilibrium, altered activity level, and abnormal respiratory behaviour are not merely academic curiosities - they translate directly into reduced predator avoidance, impaired foraging, and lower reproductive success, with knock-on effects at the population level [30, 31]. Quantifying the proportion of affected individuals across a concentration gradient gives these observations an objective, comparable footing [32, 33]. This matters because field concentrations of monocrotophos in agricultural runoff have been reported anywhere from 0.01 to 5.0 ppm depending on application intensity and local hydrology [34, 35] - a range wide enough that toxicity data are needed across it, and across more than just a 96-hour window.

Study Objectives

This study was designed around four specific aims:

1. Determine 7-day and 14-day LC_{50} values for monocrotophos in *O. mossambicus* using a static-renewal bioassay, to characterise how toxicity shifts with exposure duration.

2. Characterise the dose-response relationship through Probit analysis, comparing regression slopes across the two exposure periods.

3. Systematically document behavioural alterations - erratic swimming, loss of balance, increased mucus secretion, and reduced responsiveness to stimuli - as a function of both concentration and time.

4. Evaluate whether concentration- and time-dependent behavioural impairment can serve as an early warning signal of sublethal neurotoxicity at environmentally realistic exposure levels.

Together, these objectives are intended to sharpen the evidence base for ecological risk assessment and to inform regulatory thinking around monocrotophos use near aquatic habitats.

MATERIALS AND METHODS

Test Organism and Acclimatisation

Healthy *Oreochromis mossambicus* were sourced from a commercial fish hatchery and transported to the laboratory in oxygenated polyethylene containers (dissolved oxygen maintained above 6.5 mg/L). To limit size-related variability in toxicity response, fish were selected for uniform size (total length 6.5 ± 0.8 cm; body weight 15.2 ± 1.1 g; mean ± SD, n = 300). On arrival, fish were moved into 200-L glass aquaria filled with dechlorinated tap water that had been aged for 48 h under vigorous aeration, and allowed a 14-day acclimatisation period before any testing began.

Throughout acclimatisation, water quality was held within the ranges known to suit *O. mossambicus*: pH 7.2 ± 0.2, temperature 28 ± 2 °C, dissolved oxygen 6.5-7.8 mg/L, total hardness 180-200 mg/L as $CaCO_3$, and ammonia below 0.02 mg/L, with daily monitoring via a multiparameter probe. Fish received commercial pellet feed (35% crude protein; Sanchar Feeds, India) twice daily at 3% body weight, under a 12:12 h light:dark cycle maintained by programmable timers for the duration of both acclimatisation and testing. Only fish that were actively swimming and free of visible lesions, parasites, or abnormal behaviour were used in the bioassays. Feeding was withheld for 24 h before each bioassay began, both to standardise metabolic state across individuals and to limit ammonia build-up during the exposure period.

Test Chemical and Concentration Preparation

Technical-grade monocrotophos, supplied with a certificate of analysis, was obtained from a certified vendor. A 1000 ppm stock solution was prepared by dissolving the required quantity in distilled water with 30 min of gentle stirring to ensure complete dissolution. Working concentrations were prepared fresh by serial dilution of this stock in dechlorinated tap water immediately before each use. All glassware was acid-washed (10% HNO_3, 24 h) and rinsed three times in distilled water before solution preparation.

Bioassay Design

Toxicity testing followed the static-renewal method of Doudoroff et al., with minor modifications. Test chambers were 40-L glass aquaria (60 × 30 × 25 cm), each holding 30 L of test solution. Ten fish were placed in each aquarium as one replicate, with three replicate aquaria per concentration, giving a total of 30 fish per concentration (n = 30).

Based on preliminary range-finding trials, two separate bioassay series were run:

7-day bioassay: 0 (control), 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0 ppm (11 nominal concentrations).

14-day bioassay: 0 (control), 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, and 4.5 ppm (10 nominal concentrations).

To keep nominal concentrations and water quality reasonably stable over the exposure period, test solutions were fully renewed every 48 h. During each renewal, fish were transferred with soft nylon nets into holding containers (20 L) filled with aged water at matching temperature, the aquaria were cleaned of faeces and debris, fresh test solution was prepared, and fish were returned to their tanks within 15 minutes. This renewal cycle limits the build-up of metabolic waste without letting pesticide concentrations drift too far from nominal values. pH, temperature, and dissolved oxygen were checked daily in every test aquarium at a fixed time (09:00 h) to control

for diurnal variation. Consistent with standard acute-toxicity practice, fish were not fed during the exposure period itself.

Mortality Assessment

Fish were checked at 24-h intervals across both the 7-day and 14-day exposure periods. Mortality was scored when opercular movement had stopped completely for more than 3 minutes and the fish showed no response to gentle mechanical stimulation with a glass rod. Dead fish were removed promptly with soft nylon nets to prevent any deterioration of water quality in the remaining tank. Cumulative mortality was logged for each concentration at every observation point. Control mortality was zero throughout both series, so Abbott's correction was not required.

Behavioural Observations

Behavioural change was tracked as a sensitive, sublethal indicator of neurotoxicity, using a systematic observation protocol on days 0 (pre-exposure baseline), 1, 3, 7, and 14, always within the same window (10:00-12:00 h) to reduce diurnal noise in the data.

At each observation session, fish in every replicate tank (n = 10 fish per tank, 3 tanks per concentration) were watched for 10 minutes following a 2-minute settling period to reduce handling stress. Lighting was held constant to standardise visual conditions, and the observer worked from a fixed position 1 m from the tank without moving during the session. Tank identity was coded so the observer remained blind to treatment, reducing the risk of unconscious bias in scoring. For each session, the percentage of fish in a given tank showing each behavioural endpoint was recorded.

Six behavioural endpoints were tracked:

1. Erratic swimming rapid, uncoordinated darting with sudden, unpredictable changes in direction or speed, occurring three or more times within the 10-minute window.
2. Loss of balance (equilibrium loss)- inability to hold a normal upright posture: tilting beyond 45° from vertical, rolling, or sideways drifting sustained for at least 30 seconds.
3. Increased mucus secretion - a visibly thickened, opaque slime coating on the body and gills, clearly distinguishable from the normal mucus layer.
4. Reduced response to stimuli - a delayed (>5 s) or absent response to a standardised mechanical stimulus (a single tap on the tank wall) or visual stimulus (a shadow passed over the tank).
5. Rapid opercular movements - gill-cover beat frequency exceeding 80 beats per minute, against a control baseline of 40-50 bpm.
6. Frequent surfacing - five or more surface air-gulping events within the 10-minute observation period.

For each endpoint, time point, and concentration, the percentage of affected fish was calculated per replicate tank, and the three replicate values were averaged (mean ± SD) to give the concentration-level estimate.

Statistical Analysis

Mortality data and LC_{50} determination. Mortality data were analysed by Probit regression following Finney [43] to derive LC_{50} values and their 95% confidence intervals. Log_{10}-transformed concentrations were regressed against Probit-transformed mortality percentages to generate the dose-response relationship, from which slope ± SE, y-intercept, correlation coefficient (r), and chi-square (χ^2) goodness-of-fit statistics were obtained. LC_{50} values were treated as significantly different between the 7-day and 14-day series where their 95% confidence intervals did not overlap.

Behavioural data. Behavioural results were summarised as mean percentage ± SD across the three replicate tanks for each concentration and time point. Concentration- and time-dependent trends were evaluated descriptively, comparing behavioural frequencies across control, 2.0 ppm, and 3.0 ppm at days 1, 3, 7, and 14.

Ethical Considerations

All procedures involving live fish followed institutional guidelines for animal experimentation, and handling was carried out to minimise stress and suffering at every stage. Dead or moribund fish were removed without delay and euthanised by an overdose of tricaine methanesulfonate (MS-222, 300 mg/L), the approved method under the institutional protocol. Surviving fish at the end of each experiment were euthanised by the same method and disposed of according to institutional biosafety procedures.

RESULTS

Mortality Patterns and Dose-Response Relationships

Mortality in O. mossambicus exposed to monocrotophos rose in a clearly concentration- and time-dependent manner (Table 1; Figure 1 presents the cumulative mortality curves and sigmoid dose-response plots for both exposure periods). No mortality occurred in either control group across the full 7- or 14-day period, which rules out baseline water quality or handling stress as a confound in the toxicity data.

Table 1. Cumulative percentage mortality of *O. mossambicus* (n = 30 per concentration) exposed to monocrotophos over 7- and 14-day periods. Δ Mortality is the percentage-point increase from 7 to 14 days at matching concentrations. Concentrations of 6.0-10.0 ppm in the 7-day series produced 100% mortality within 3-5 days. Dashes indicate concentrations not tested in that series.

Concentration (ppm)	7-Day Mortality (%)	14-Day Mortality (%)	Δ Mortality (%)
0.0 (Control)	0	0	0
0.5	-	10	-
1.0	10	30	+20
1.5	-	45	-
2.0	30	60	+30
2.5	-	75	-
3.0	70	90	+20
3.5	-	95	-
4.0	90	100	+10
4.5	-	100	-
5.0	100	100	0

In the 7-day series, mortality climbed steadily from 10% at 1.0 ppm to complete mortality at 5.0 ppm and above, tracing the sigmoid shape typical of a well-behaved dose-response curve. The 14-day series showed consistently higher mortality at every matched concentration: at 1.0 ppm, mortality rose from 10% to 30% between the two durations; at 2.0 ppm, from 30% to 60%; and at 3.0 ppm, from 70% to 90%. The size of this shift - 10 to 30 percentage points depending on concentration - makes clear that duration of exposure, and not just concentration, is doing meaningful work in determining outcome.

Probit Analysis and LC_{50} Determination

Probit modelling produced stable, well-fitted LC_{50} estimates for both exposure windows (Table 2). The 7-day LC_{50} was 3.6 ppm (95% CI: 3.2-4.1 ppm); by 14 days this had fallen to 2.4 ppm (95% CI: 2.1-2.7 ppm), a 33% decline. Because the confidence intervals for the two estimates do not overlap, this shift can reasonably be treated as a real effect of exposure duration rather than sampling noise.

Table 2. Probit analysis parameters for LC_{50} estimation in *O. mossambicus* exposed to monocrotophos. CI= confidence interval; SE= standard error; r = Pearson correlation coefficient; χ^2 = chi-square goodness-of-fit statistic; p > 0.05 indicates acceptable model fit.

Exposure Period	LC_{50} (ppm)	95% CI (ppm)	Slope ±SE	r	χ^2	p-value
7-Day	3.6	3.2-4.1	4.2 ± 0.6	0.96	2.8	0.42
14-Day	2.4	2.1-2.7	5.1 ± 0.8	0.97	1.9	0.59

The steeper slope of the 14-day model (5.1 versus 4.2) indicates a sharper dose-response relationship at the longer duration: as exposure lengthens, a given increment in concentration produces a proportionally larger increase in mortality. Both models fit the data well ($\chi^2 = 2.8$, $p = 0.42$ for 7 days; $\chi^2 = 1.9$, $p = 0.59$ for 14 days), which lends confidence to the LC_{50} estimates derived from them.

Behavioural Alterations

Control fish behaved normally across both exposure windows- smooth, coordinated swimming, a steady opercular rate around 40-50 beats per minute, normal balance, and no visible signs of stress. Fish exposed to 1.0 ppm monocrotophos or higher, by contrast, showed clear behavioural disturbance that intensified with both concentration and duration (Table 3).

Table 3. Percentage of fish (mean ± SD; n = 3 replicate tanks of 10 fish each) displaying behavioural alterations at selected monocrotophos concentrations during 7- and 14-day exposures. Control fish showed 0% incidence for all endpoints throughout the study. Data reflect observations from surviving fish at each time point.

Behavioral Endpoint	7-Day: 2.0 ppm (%)	7-Day: 3.0 ppm (%)	14-Day: 2.0 ppm (%)	14-Day: 3.0 ppm (%)
Erratic swimming	35 ± 8	70 ± 6	55 ± 7	85 ± 5
Loss of balance	25 ± 6	60 ± 7	45 ± 8	75 ± 6
Increased mucus secretion	15 ± 5	40 ± 6	30 ± 6	60 ± 7
Reduced response to stimuli	20 ± 7	55 ± 8	40 ± 7	70 ± 6
Rapid opercular movements	30 ± 6	65 ± 7	50 ± 8	80 ± 6
Frequent surfacing	25 ± 7	50 ± 6	45 ± 7	70 ± 7

Initial phase (24-48 h). The earliest visible change was heightened activity - fish swam irregularly and restlessly, with sudden bursts of movement and unpredictable direction changes. At 2.0 ppm, around 35% of fish showed this erratic swimming by day 7, rising to 55% by day 14; at 3.0 ppm the pattern was more pronounced still, affecting 70% of fish by day 7 and 85% by day 14 (Table 3). This early response, appearing within the first 24-48 hours across the 2.0-4.0 ppm range, is consistent with cholinergic overstimulation following AChE inhibition.

Progressive phase (days 3-7). As exposure continued, the initial hyperactivity gave way to more clearly pathological signs. Fish began struggling to hold an upright posture, with tilted swimming beyond 45° and occasional rolling. At 2.0 ppm, loss of balance affected 25% of fish by day 7 and 45% by day 14; at 3.0 ppm the figures were 60% and 75%, respectively (Table 3) - a pattern suggesting a steady erosion of neuromuscular coordination over the course of exposure.

Responsiveness to external stimuli declined over the same window. At 3.0 ppm, 55% of fish showed reduced reaction to visual or mechanical stimuli by day 7, climbing to 70% by day 14; at 2.0 ppm the effect was present but milder (20% at day 7, 40% at day 14). Diminished responsiveness of this kind points to impairment on both the sensory and motor sides of the toxic response.

Advanced phase (days 7-14). In fish that survived into the later stage of exposure, signs of respiratory and physiological stress became more prominent. Rapid opercular movement (>80 bpm) was seen in 65% of fish at 3.0 ppm by day 7 and 80% by day 14; frequent surfacing -five or more air-gulping events in 10 minutes- affected 50% of fish at day 7 and 70% at day 14. Excessive mucus secretion followed a similar trajectory, affecting 40% of fish at day 7 and 60% at day 14 at 3.0 ppm (versus 15% and 30% at 2.0 ppm). Together, these patterns are consistent with gill irritation, disrupted respiratory control, and reduced gas exchange efficiency as mucus accumulates on the respiratory surfaces.

Concentration-Dependent and Time-Dependent Patterns

Figure 2. illustrates that every behavioural endpoint worsened as concentration and exposure duration increased together. At 2.0 ppm, effects were moderate - roughly 15-35% of fish affected by day 7. At 3.0 ppm, the impact was substantially larger, with 40-70% of fish affected over the same window, and this pattern held consistently across all six endpoints.

A clear time-dependent trend accompanied the concentration effect. At 3.0 ppm, for example, erratic swimming rose from 70% at day 7 to 85% at day 14, and loss of balance rose from 60% to 75% over the same interval - a trajectory that mirrors the decline in LC_{50} between the two exposure windows and reinforces the idea that toxicity is compounding over time rather than remaining static. At 4.0 ppm and above, fish typically displayed several of these symptoms simultaneously in the period shortly before death.

DISCUSSION

Time-Dependent Toxicity and Bioaccumulation

The 33% drop in LC_{50} between 7 and 14 days is itself the study's central finding, and it points fairly directly to a compounding toxicity that acute assays are not built to see [24]. Fish appear able to tolerate monocrotophos reasonably well in the short term, but that tolerance seems to erode as exposure continues - consistent with a system whose compensatory capacity is being progressively used up [25]. One plausible explanation is that fish can partially offset organophosphate toxicity by synthesising new AChE to replace the inhibited enzyme, but that this compensatory synthesis becomes harder to sustain under continued exposure, and its energetic cost compounds alongside accumulating neurotoxic damage [44, 45].

The behavioural data are broadly consistent with this picture. Surfacing frequency and mucus secretion, in particular, worsened over time in a way that aligns with gill damage reported in other chronic-exposure studies [46, 47], while the shift from early hyperactivity to later respiratory and postural distress reads as a transition from acute nervous system overstimulation to something closer to systemic physiological failure [30].

Taken together, the decline from a 3.6 ppm LC_{50} at 7 days to 2.4 ppm at 14 days is a fairly direct demonstration that duration matters as much as concentration in this system - and a reminder that a single 96-hour endpoint is likely to understate the risk that prolonged field exposure actually poses.

Comparative Toxicity Assessment

Placing these LC_{50} values against the existing literature is instructive. Previously reported 96-hour LC_{50} values for *O. mossambicus* range from 0.36 to 1.15 ppm [20, 48, 49], noticeably lower than the 7-day LC_{50} of 3.6 ppm obtained here. Some of that gap is likely attributable to differences in experimental conditions - fish size and developmental stage, water temperature, and hardness are all known to shift toxicity outcomes [50, 51] - though it is also worth noting that a longer exposure duration does not automatically translate into a proportionally lower

LC_{50} at every step; the relationship depends on how quickly a species can mount and sustain a compensatory response.

In *Heteropneustes fossilis*, reported 96-hour LC_{50} values (2.1-3.8 ppm) suggest a sensitivity roughly comparable to that of *O. mossambicus* [52]. Zebrafish (*Danio rerio*), by contrast, appear considerably more sensitive, with LC_{50} values reported between 0.28 and 0.45 ppm [8]. These interspecies gaps most likely reflect differences in uptake rate, detoxification capacity, and target-site sensitivity to organophosphates [53, 54], and they are a useful reminder that no single species can stand in for the full range of ecological risk. A multi-species testing approach - even if more resource-intensive - gives a more defensible basis for setting water quality guidelines than reliance on one model organism alone.

Behavioural Responses as Indicators of Neurotoxicity

The behavioural sequence documented here - early hyperactivity, followed by equilibrium loss, followed by respiratory distress - tracks closely with what has been reported elsewhere for organophosphate-exposed fish [28, 37, 55], which lends some confidence that this is a general feature of the exposure rather than an artefact specific to this study. The initial hyperactivity is readily explained by acetylcholine accumulation following AChE inhibition [10]; the subsequent loss of equilibrium points to a breakdown in neuromuscular coordination [37, 38]; and the later respiratory symptoms - rapid opercular movement, frequent surfacing - are consistent with gill irritation or mucus-driven impairment of gas exchange [39, 46].

What stands out is that these behavioural changes were present at concentrations well below the LC_{50}. That is precisely the value of behavioural monitoring as a toxicological tool: it captures functional impairment - reduced ability to avoid predators, forage effectively, or track environmental cues - that a mortality-only endpoint would simply miss, even though that impairment can still carry real consequences for survival in the wild [26, 56, 57].

Implications for Environmental Risk Assessment

These findings carry a fairly direct message for how monocrotophos is regulated near aquatic systems. The gap between the 7-day and 14-day LC_{50} (3.6 vs. 2.4 ppm) is large enough to suggest that standard 96-hour assays are likely underestimating toxicity under the kind of prolonged, low-level exposure that actually characterises agricultural runoff [23, 58].

Field contamination rarely resembles a single acute dose; it is closer to a repeated or continuous low-level input driven by runoff and irrigation return flow [34]. Reported monocrotophos concentrations in such waters - 0.01 to 5.0 ppm [4, 35] - comfortably bracket the 14-day LC_{50} of 2.4 ppm obtained here, which suggests that fish populations in genuinely contaminated water bodies may face a tangible risk over sustained exposure periods, not merely a hypothetical one confined to the laboratory.

Behavioural disturbance, moreover, was evident at concentrations as low as 1.0-2.0 ppm - well under the LC_{50}. At 2.0 ppm, roughly 25-35% of fish showed behavioural impairment by day 7, rising to 30-55% by day 14. None of this is immediately lethal, but it is disruptive enough to feeding, predator avoidance, and general environmental responsiveness that a risk framework built solely around LC_{50} and a conventional safety factor is likely to miss it. Incorporating extended exposure windows and behavioural endpoints into standard risk assessment protocols would go some way toward closing that gap [29, 59]. This also sits consistently with the regulatory direction several jurisdictions have already taken - monocrotophos has been banned or heavily restricted in the European Union and the United States on toxicity grounds - even as it remains in active use in a number of developing regions where the trade-off between agricultural need and environmental protection is still being negotiated.

Study Limitations and Future Research Directions

A few limitations are worth flagging plainly. First, this was a controlled laboratory study, and laboratory conditions cannot fully reproduce the variability of a natural water body - fluctuating pesticide concentrations, temperature swings, dissolved oxygen variation, co-occurring contaminants, predation pressure, and social

behaviour all interact in ways that a static tank cannot capture [50]. Field validation - tracking AChE activity, tissue condition, and behaviour in fish sampled from genuinely contaminated water bodies - would go a long way toward confirming that the laboratory findings hold up under real conditions.

Second, the study used a single species, which limits how far these results can be generalised across aquatic taxa [19]. Testing across multiple species and trophic levels would give a fuller picture of ecosystem-level risk.

Third, the absence of biochemical data is a real gap. Direct measurement of AChE activity in brain and muscle tissue would allow enzyme inhibition to be quantified and tied more precisely to the behavioural and mortality data reported here. As a general reference point, prior work suggests that behavioural disturbance in organophosphate-exposed fish tends to emerge once AChE inhibition reaches roughly 50-80%, which offers a useful benchmark for interpreting the present results once biochemical data become available.

Histopathological analysis would add a further layer of mechanistic support - lamellar fusion or oedema in gill tissue would corroborate the respiratory distress observed here, liver and kidney changes would speak to detoxification burden and systemic toxicity, and any brain tissue changes would provide direct evidence for the neurological basis of the balance and responsiveness deficits documented above [60]. Oxidative stress markers (malondialdehyde, superoxide dismutase, catalase, reduced glutathione) would help clarify secondary mechanisms that may be contributing to toxicity as exposure lengthens. Folding these biochemical and histological measures into future work would substantially strengthen the mechanistic case built here.

Beyond these immediate gaps, several broader directions merit attention:

1. Biochemical biomarker integration - AChE activity, oxidative stress indices, and histopathology of brain, gill, liver, and kidney tissue [14, 61].

2. Chronic toxicity and recovery studies - exposure periods of 28 days or longer, followed by depuration in clean water, to characterise bioaccumulation kinetics, chronic-effect thresholds, and the reversibility (or otherwise) of behavioural and physiological impairment [62].

3. Multiple-stressor experiments - combining monocrotophos exposure with other environmental stressors (elevated temperature, hypoxia, ammonia, co-occurring pesticides) to approximate the interactive effects likely under field conditions [51].

4. Early life-stage sensitivity - developmental toxicity testing in embryos and larvae, which typically lack fully developed detoxification pathways and may be disproportionately vulnerable, with implications for population-level recruitment [62].

5. Field validation studies - monitoring AChE activity, histopathology, and behaviour in fish sampled from runoff-affected water bodies, to test whether laboratory-derived effect concentrations hold up under field conditions [18].

6. Bioremediation approaches - exploring microbial degradation pathways and the phytoremediation potential of aquatic macrophytes as a means of reducing monocrotophos loads in agricultural drainage [63, 64].

Regulatory and Management Implications

Taken as a whole, the evidence here supports several concrete recommendations:

1. Extended bioassay protocols. Regulatory testing guidelines for organophosphates and other pesticides with cumulative or bioaccumulative mechanisms should incorporate 7-day or 14-day exposure windows alongside the standard acute assay, to produce toxicity estimates that better reflect field exposure [58].

2. Behavioural endpoints as standard practice. Standardised behavioural assessment protocols deserve a formal place in pesticide risk assessment frameworks, since they capture sublethal, ecologically relevant effects that mortality-based testing simply cannot [33].

3. Species sensitivity distributions. Building out toxicity databases across multiple fish species would support the development of species sensitivity distributions (SSDs) and more statistically defensible, probabilistic water quality criteria [54].

4. Buffer zones and best management practices. Vegetated buffer strips, constructed wetlands for runoff treatment, and controlled drainage systems should be part of standard agricultural practice near sensitive water bodies, to reduce the volume of pesticide reaching aquatic systems in the first place [18, 34].

5. Integrated pest management. Reducing reliance on organophosphates through IPM strategies - biological control, resistant crop varieties, crop rotation, and precision application - offers a route to lowering environmental contamination without sacrificing agricultural output [65].

6. Ongoing monitoring. Regular surveillance of monocrotophos levels in surface water, sediment, and aquatic biota in agricultural regions would help track compliance with water quality standards, flag high-risk locations, and gauge whether management interventions are actually working.

CONCLUSIONS

This study shows that monocrotophos toxicity in *Oreochromis mossambicus* rises significantly with exposure duration, reflected in a 33% decline in LC_{50} from 3.6 ppm at 7 days to 2.4 ppm at 14 days. That gap alone is evidence that short-duration toxicity tests risk underestimating real-world hazard where exposure is prolonged. Behaviourally, the toxic response followed a fairly consistent arc - beginning with erratic swimming, progressing to loss of equilibrium and reduced responsiveness, and culminating in respiratory distress and elevated mucus secretion. Critically, several of these effects were detectable at concentrations below the LC_{50}, which is precisely what makes them useful as early, sensitive indicators of sublethal neurotoxicity.

Both mortality and behavioural impairment showed strong concentration- and time-dependent trends, and the effect levels observed here overlap with concentrations already reported in agricultural runoff - which suggests this is not merely a laboratory phenomenon but a plausible risk for fish populations in contaminated water bodies.

On balance, these findings make a reasonably strong case for building longer exposure periods and behavioural endpoints into standard toxicity assessment, and for pairing that scientific shift with stronger regulatory frameworks and better agricultural management practices to limit the reach of organophosphate pesticides into aquatic ecosystems.

Compliance with Ethical Standards

Conflict of Interest: The author declares no conflicts of interest.

Ethical Approval: All experimental procedures involving fish were conducted in accordance with institutional ethical guidelines for animal research.

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